

IN THE CLAIMS:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims

1. (Currently Amended) A chimeric nucleic acid comprising a GAP promoter domain, a first domain comprising a yeast Pir (protein internal repeat) cell wall protein coding sequence and a second domain comprising a peptide or a polypeptide coding sequence, wherein the GAP promoter domain is operably linked to the first and second domain and wherein the a yeast cell wall protein expressed from the chimeric nucleic acid is capable of being localized or immobilized on a yeast cell wall.
2. (Currently Amended) The chimeric nucleic acid of claim 1, wherein the yeast cell wall protein comprises a Pir (protein internal repeat) motif coding sequence a yeast Pir (protein internal repeat) cell wall protein coding sequence.
3. (Currently Amended) The chimeric nucleic acid of claim 2, wherein the yeast Pir (protein internal repeat) cell wall protein motif coding sequence comprises an amino acid sequence as set forth by SEQ ID NO: 1 or SEQ ID NO: 2.
4. (Currently Amended) The chimeric nucleic acid of claim 2, wherein the yeast Pir (protein internal repeat) cell wall protein motif coding sequence comprises a protein comprising an amino acid sequence derived from an amino acid sequence as set forth by SEQ NO: 1 by deletion, replacement, or addition of one or more amino acids of SEQ NO: 1, wherein the yeast Pir (protein internal repeat) cell wall protein motif coding sequence is capable of being localized or immobilized on a yeast cell wall.

5. (Original) The chimeric nucleic acid of claim 1, wherein the polypeptide is an enzyme.
6. (Currently Amended) The chimeric nucleic acid of claim 4-5, wherein the enzyme is a glycosyltransferase.
7. (Currently Amended) The chimeric nucleic acid of claim 4-2, wherein the yeast Pir (protein internal repeat) cell wall protein motif coding sequence is located 5' to the peptide or a polypeptide coding sequence.
8. (Currently Amended) An expression cassette comprising a chimeric nucleic acid comprising a GAP promoter domain, a first domain comprising a Pir (protein internal repeat) cell wall protein motif coding sequence and a second domain comprising a peptide or a polypeptide coding sequence, wherein the second domain encodes an enzyme and wherein the GAP promoter domain is operably linked to the first and second domain.
9. (Currently Amended) ~~The~~ An expression vector comprising the expression cassette of claim 8 comprising an expression vector.
10. (Currently Amended) The expression ~~cassette~~ vector of claim 9, wherein the expression vector ~~comprises~~ is a yeast expression vector.
11. (Currently Amended) A host cell comprising an expression cassette comprising a chimeric nucleic acid comprising a GAP promoter domain, a first domain comprising a yeast Pir (protein internal repeat) cell wall protein motif coding sequence and a second domain comprising a peptide or a polypeptide coding sequence, wherein the second domain encodes an enzyme and wherein the GAP promoter domain is operably linked to the first and second domain.

12. (Currently Amended) The host cell of claim 11 ~~comprising a yeast cell~~, wherein the host cell is a yeast host cell.

13. (Original) The host cell of claim 11 comprising a yeast cell wall.

14. (Currently Amended) An expression vector comprising a fusion gene comprising a nucleic acid encoding ~~a useful~~ an enzyme protein downstream of a nucleic acid encoding a yeast cell wall protein selected from the group consisting of (a) a protein having an amino acid sequence represented by SEQ ID NO: 1, and (b) a protein comprising an amino acid sequence derived from an amino acid sequence as set forth by SEQ NO: 1 by deletion, replacement, or addition of one or more amino acids of SEQ NO: 1 having at least 80% identity to SEQ ID NO:1, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall and wherein the nucleic acid encoding the yeast cell wall protein is downstream and operably linked to a GAP promoter domain.

15. (Currently Amended) The expression vector of claim 14, wherein the ~~useful~~ enzyme protein is a glycosyltransferase protein.

16. (Currently Amended) A transformant yeast transformed by an expression vector, wherein the expression vector comprises a chimeric nucleic acid comprising a nucleic acid encoding ~~a useful~~ an enzyme protein downstream of a nucleic acid encoding a yeast cell wall protein selected from the group consisting of (a) a protein having an amino acid sequence represented by SEQ ID NO: 1, and (b) a protein comprising an amino acid sequence derived from an amino acid sequence as set forth by SEQ NO: 1 by deletion, replacement, or addition of one or more amino acids of SEQ NO: 1, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall and wherein the nucleic acid encoding the yeast cell wall protein is downstream and operably linked to a GAP promoter domain.

17. (Withdrawn) A chimeric polypeptide comprising a first domain comprising a yeast cell wall protein and a second domain comprising a peptide or a polypeptide of interest, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall.

18. (Withdrawn) A particle comprising a chimeric polypeptide comprising a first domain comprising a yeast cell wall protein and a second domain comprising a peptide or a polypeptide of interest, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall component, and a yeast cell wall component.

19. (Withdrawn) The particle of claim 18, wherein the particle is a resin.

20. (Withdrawn) A solid support comprising a chimeric polypeptide comprising a first domain comprising a yeast cell wall protein and a second domain comprising a peptide or a polypeptide of interest, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall component, and a yeast cell wall component.

21. (Withdrawn) The solid support of claim 20, wherein the solid support comprises a tube, a fiber, a plate or a filter.

22. (Currently Amended) A method for producing an immobilized polypeptide comprising the following steps:

(a) providing an expression vector, wherein the expression vector comprises a chimeric nucleic acid encoding a fusion polypeptide, wherein the chimeric nucleic acid comprises a nucleic acid encoding ~~a useful~~ an enzyme protein downstream of a nucleic acid encoding a yeast cell wall protein selected from the group consisting of (a) a protein having an amino acid sequence represented by SEQ ID NO: 1, and (b) a protein comprising an amino acid sequence derived from an amino acid sequence as set forth by SEQ NO: 1 by deletion, replacement, or

addition of one or more amino acids of SEQ NO: 1 having at least 80% identity to SEQ ID NO:1, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall and wherein the nucleic acid encoding the yeast cell wall protein is downstream and operably linked to a GAP promoter domain;

(b) transforming ~~a microorganism comprising~~ a yeast cell wall with the expression vector of step (a);

(~~b c~~) culturing the ~~transformant microorganism~~ transformed yeast cell of step (b) and expressing the fusion polypeptide on a surface layer of the yeast cell wall, thereby producing an immobilized polypeptide.

23. (Currently Amended) The method of claim 22, wherein the ~~useful~~ enzyme protein is a glycosyltransferase protein.

24. (Currently Amended) The method of claim 22, wherein the microorganism ~~comprises~~ is a yeast.

25. (Withdrawn) An immobilized enzyme obtained by the method of claim 22.

26. (Withdrawn) The immobilized enzyme of claim 25, wherein the enzyme is a glycosyltransferase.

27. (Withdrawn) A method for producing a sugar chain or a sugar comprising use of an immobilized enzyme as set forth in claim 22.

28. (Currently Amended) A method for producing an immobilized enzyme on the surface of a yeast cell comprising culturing the ~~host yeast~~ cell of claim 12 and obtaining a yeast comprising ~~a useful the enzyme protein~~ immobilized on its the yeast's cell wall.

29. (Withdrawn) An immobilized enzyme obtained by the method of claim 28.

30. (Withdrawn) The immobilized enzyme of claim 29, wherein the enzyme immobilized is a glycosyltransferase.

31. (Withdrawn) A method for producing a sugar chain or a sugar which employs the immobilized enzyme of claim 29.

32. (Original) A transformant yeast which is transformed by allowing the yeast to comprise an expression cassette as set forth in claim 8 or an expression vector as set forth in claim 14.

33. (Currently Amended) A method for producing an immobilized enzyme which comprises the steps of:

- (a) culturing the transformant yeast of claim 32,
- (b) expressing chimeric polypeptides on the surface layer of a cell wall of the transformant yeast, and
- (c) isolating a transformant yeast that expresses a chimeric polypeptide immobilized on the cell wall.

34. (Withdrawn) An immobilized enzyme obtained by the method of claim 33.

35. (Withdrawn) The immobilized enzyme of claim 34, wherein the enzyme immobilized is a glycosyltransferase.

36. (Withdrawn) A method for producing a sugar chain or a sugar, wherein the method comprises sequentially converting a sugar chain or a sugar using an immobilized enzyme as set forth in claim 34.

37. (Currently Amended) A chimeric nucleic acid comprising a GAP promoter domain, a first domain comprising ~~a yeast cell wall protein coding sequence~~ a yeast Pir (protein internal repeat) cell wall protein coding sequence and a second domain comprising an enzyme coding sequence, wherein the GAP promoter domain is operably linked to the first and second domain and wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall and the enzyme is selected from the group consisting of a fucosyltransferase, a ~~Lacto-N-fucopentaose~~ a mannosyltransferase, a galactosamyltransferase, a sialyltransferase, a N-acetylglucosamyltransferase, a galactosyltransferase, and a glucosyltransferase.

38. (Withdrawn) A chimeric polypeptide comprising a first domain comprising a yeast cell wall protein and a second domain comprising an enzyme, wherein the yeast cell wall protein is capable of being localized or immobilized on a yeast cell wall and the enzyme is selected from the group consisting of a fucosyltransferase, a Lacto-N-fucopentaose, a galactosyltransferase, and a glucosyltransferase.